CELL DIVISION SYNCHRONIZATION

Final Report

by

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April 15, 1972

The research was supported by the Office of Naval Research through Contract N00014-69-A-0206-0001, under Project Number NR 306-008

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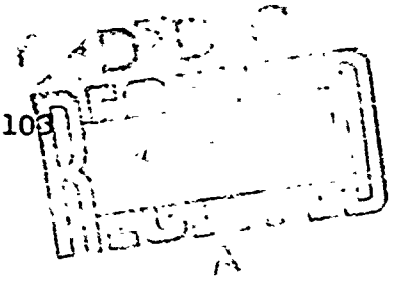

Security Classification

DOCUMENT CONTROL DATA - R & D

Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified

1 ORIGINATING ACTIVITY (Corporate author)	2a. REPORT SECURITY CLASSIFICATION
Department of Biology St. Louis University St. Louis, Missouri 63074	Unclassified
	2b. GROUP

3 REPORT TITLE

Cell Division Synchronization

4 DESCRIPTIVE NOTES (Type of report and inclusive dates)

Final report

5 AUTHOR(S) (First name, middle initial, last name)

David W. Rooney, Ph.D.

6 REPORT DATE	7a. TOTAL NO OF PAGES	7b. NO OF REFS
April 15, 1972	3	6

8a CONTRACT OR GRANT NO	9a. ORIGINATOR'S REPORT NUMBER(S)
N00014-69-A-0206-0001	
b PROJECT NO	#2
c	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d.	

10 DISTRIBUTION STATEMENT

Distribution of this document is unlimited.

11 SUPPLEMENTARY NOTES	12 SPONSORING MILITARY ACTIVITY
	U. S. Office of Naval Research

13 ABSTRACT

Reported is progress in the design and construction of automatic equipment for synchronizing cell division in culture by periodic hypoxia; problems and progress in testing appropriate carbohydrate sources for human cells in suspension cultures to be subjected to periodic hypoxia. Concurrent experiments in hypothermic synchronization of algal cell division are reported.

DD FORM 1 NOV 65 1473 (PAGE 1)

S/N 0101-807-6801

Security Classification

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Date: March 15, 1972

Subject: Research under contract N0014-69-A-0206-0001

FINAL REPORT

The specific objective at the onset of research under N0014-69-A-0206-0001 was to determine if periodic hypoxia is a suitable method for synchronizing division in cell cultures. The basic underlying purpose was to establish an additional useful biophysical technique for the study of the division cycle of eukaryotic (nucleated) cells.

A portion of the research has consisted in the design, testing, and refinement of automatic equipment to impose upon cell cultures any desired cyclic program of oxygen concentration. A preliminary description of this equipment may be found in a technical report (2) submitted to ONR. Subsequent refinements, such as nylon inserts to speed O_2 concentration changes have been made.

Another portion of the research has consisted of testing various sugars (glucose, fructose, galactose) at various concentrations in human (HeLa) cell suspension cultures. This testing was done to determine if rapid division (growth) rates of mammalian cells in suspension culture can be achieved with low glycolytic rates. Only when glycolysis is slow would oxygen deprivation be effective in limiting ATP production (since ATP is generated by both glycolysis and oxidative phosphorylation). Only when growth is rapid can biophysical perturbations (such as temporary O_2 reductions) be expected to synchronize division. Extensive testing of growth rate and pH charge (an index of glycolysis rate) with various sugars was carried out by Miss Judith O'Brien who reported the results in her Master's Thesis (2). It was found that neither fructose nor galactose permitted rapid growth in suspension cultures.

Glucose permitted rapid growth -- but with rapid glycolysis.

In the course of these experiments, it became obvious that better facilities were needed to insure the absence of bacterial contamination in the human cell cultures. For this reason experiments with human cell suspensions have been temporarily halted. They will resume this summer (August, 1972) in a specially designed cell culture laboratory in the remodeled McDonnell Building at St. Louis University. Planned experiments will include steady-state culturing at low glucose concentration and imposition of periodic hypoxia.

Concurrent with the above described experimentation have been studies of periodic hypothermia as a method of synchronizing eukaryotic cell division. Because these studies are similar in objective, they are mentioned here. Briefly, I have found that periodic temperature reductions will effectively synchronize the division of an algal cell Chlamydomonas. The resulting publication (3) credits ONR support as do three additional publications (4, 5, 6) describing appropriate mathematical techniques for quantifying cell division synchrony in algal cultures.

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